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ELECTRON MICROSCOPICAL OBSERVATIONS ON *SPIROSTOMUM AMBIGUUM*

BY
HAROLD E. FINLEY

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ELECTRON MICROSCOPICAL OBSERVATIONS ON *SPIROSTOMUM AMBIGUUM*

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Introduction

The observations described in this paper were made with the object of determining whether an electron microscopical examination of *Spirostomum ambiguum* would yield any new facts about this organism or lead to the modification of existing opinions relating to the morphology of the protozoa. This study was undertaken because:

(1) In the past, concepts concerning the organization of the protozoan cell have been dependent upon morphological data acquired with the aid of the optical microscope. Such data have broadened our understanding of biological phenomena and have given us keener insight into the maze of microscopic life to which the scientific world was first introduced by Leeuwenhoek in 1675.

(2) At present, electron microscopy is making it possible to visualize many biological structures 10 times smaller than those previously visible with the ordinary microscope.

Many investigators have studied *Spirostomum*. Indeed, this ciliated protozoan has been the subject of inquiries for more than a century. During that time, observations regarding its morphology have been reported by Dujardin,[1] Claparède and Lachmann,[2] Stein,[3] Bütschli,[4] Neresheimer,[5] Maier,[6] Pütter,[7] Wetzel,[8] and others. Accordingly, the literature contains descriptions of this heterotrich's cilia, membranelles, basal bodies, and myonemes. Also, there are reports pertaining to the cytostome or cell mouth, the cytopyge or cell anus, the contractile vacuole, macronucleus, and other vital organelles of this protozoan. The earlier authors clearly established the identity of the cytoplasmic structures named above, but the most detailed account of these structures in recent literature was published by Bishop.[9]

Wenrich's[10] review of the literature shows that, in 1947, very few inquiries stemmed from the application of electron microscopy to protozoan materials, even though, with the aid of the optical microscope, investigations of the morphology and cytology of Protozoa had been vigorously pursued. The absence of such inquiries is not surprising, however, since the application of electron microscopy to the science of cytology required the development of satisfactory techniques of specimen preparation. With the development and refinement of the techniques of

*The author is greatly indebted to the following members of the staff at the National Bureau of Standards, United States Department of Commerce, in Washington, D. C., for assistance rendered during the progress of this investigation: Sanford B. Newman, Max Swerdlow, Emil Borysko, and Barbara Sullivan.

specimen preparation have come programs of biological research designed with a view toward utilizing the electron microscope. As stated by Hillier,[11] these programs have been designed for the purpose of identifying structures within the pattern of existing knowledge or of extending existing knowledge.

Materials and Methods

Spirostomum ambiguum (Ehrenberg) is characterized by an elongated, cylindrical, slightly compressed body. Average mature specimens measure about 1500 micra in length and 150 micra in width. The animal's cilia are distributed over all surfaces and are uniformly the same, except for a peristomial band of membranelles that extend in a sinistral spiral from the anterior end to the cytostome. The cytostome is located posterior to the middle of the longitudinal axis. The large pulsating vacuole occupies the major portion of the posterior end and communicates with a narrow canal that entends to the anterior end of the body. The macronucleus resembles a string of pearls because it is composed of approximately 20 adjoined lobes. It tends to lie parallel with the longitudinal axis of the body. These diagnostic features are shown in FIGURE 1.

The specimens used in this study were obtained from cultures established in our laboratory. The original stock was taken from pond water, and its descendants have been maintained in uncovered battery jars. From time to time, the cultures have been inoculated with an infusion prepared from dried kernels of the green pea, *Pisum sativum*.

Specimen fixation. Many fixing solutions were tested in this study. It was found that Bouin's fluid and Randolph's modification of Navashin's chromic-acetic-formalin mixture gave better results than any of the fixatives that were tested. In general, plain 2 per cent osmium tetroxide solution was too unconvincing to impress the author with its effectiveness. This statement, however, is not intended to discourage the use of fixatives that contain osmic acid.

Fixation was brought about by concentrating large numbers of *Spirostoma* in a small volume of culture fluid, contained, invariably, in graduated centrifuge tubes. Following this step, the fixative was poured in and the tubes were agitated gently. The ratio of two volumes of fixative to one volume of culture fluid was rigidly observed. Bouin's fluid was heated to 50° C. immediately before it was poured into the centrifuge tubes, but Randolph's mixture, and all other fixatives, were used at room temperature. The *Spirostoma* were left in the fixative for periods of 2 to 24 hours, then the fixative was thoroughly washed out, and the animals were dehydrated through a dioxan series or through an ethyl alcohol series.

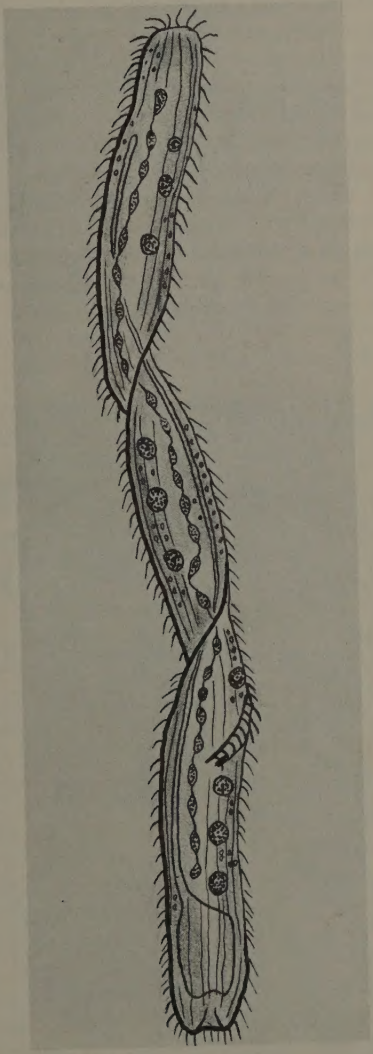

FIGURE 1. Diagrammatic drawing of *Spirostomum ambiguum*. See text for description.

Infiltration, embedding, sectioning. After dehydration, the specimens were transferred from centrifuge tubes to gelatin capsules by means of an ordinary long-shanked pipette. Subsequently, they were infiltrated with the monomer of n-butyl methacrylate, embedded, and sectioned according to the procedure developed at the National Bureau of Standards of the United States Department of Commerce, Washington, D. C., by Newman, Borysko, and Swerdlow.[12] After polymerization of the monomer had been accomplished, each resin mold contained a large number of *Spirostoma* which, during the course of encapsulation, had been oriented at random when they settled to the bottom of the gelatin capsule. Therefore, each thin section of the resin mold contained slices from the bodies of different specimens that had been cut in different ways: for example, transversely, longitudinally, or obliquely. Most of our preparations were studied with the 50-kv., RCA-type EMU electron microscope, although some specimens were studied with the RCA-type EMT.

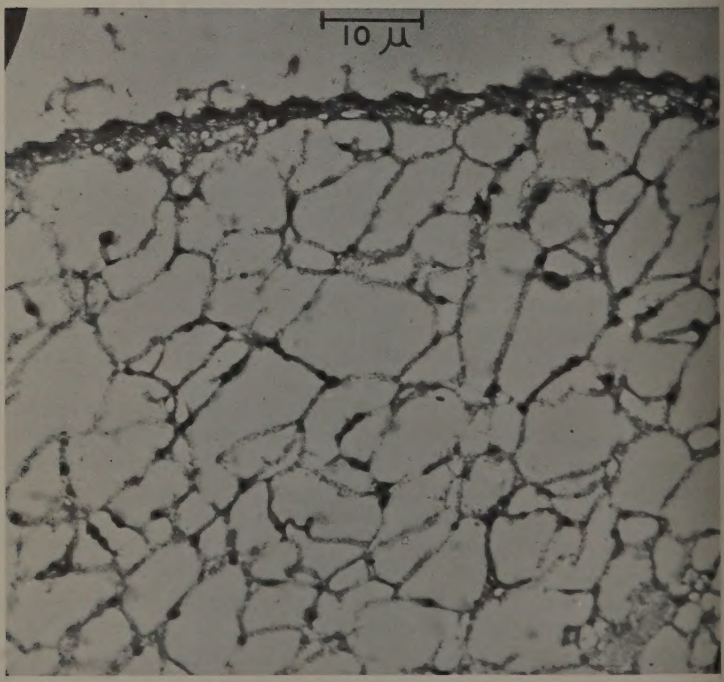

FIGURE 2. Peripheral section of ectoplasm and endoplasm. Ectoplasm characterized by ridges and furrows. Bouin's fixation.

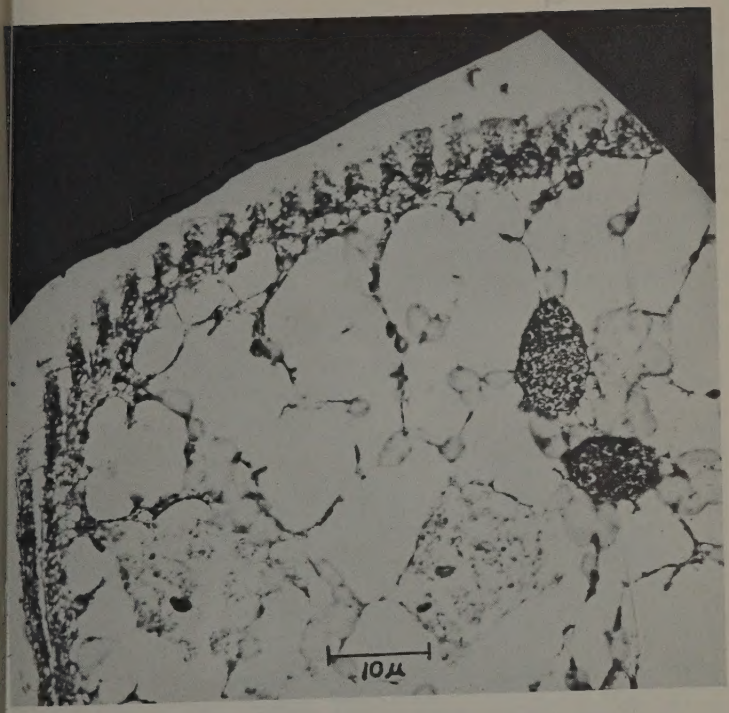

FIGURE 3. Tangential section showing ectoplasm, two opaque ovoid portions of the macronucleus, two food vacuoles, and many transparent ovoid symbionts. Bouin's fixation.

Observations

The following brief description* sets forth the pattern of existing concepts regarding the morphology of *Spirostomum* and is presented here for the purpose of orientation. The striped appearance of a living specimen is due to the presence of ridges and furrows in the ectoplasm. The outer ectoplasm is believed to be covered by a very delicate cuticle. Immediately below or embedded in the ectoplasmic furrows lie the myonemes. Rows of basal granules, from which locomotory cilia spring, lie beside or slightly above the myonemes. The peristomial membranelles are compound ciliary structures, each membranella being composed of two parallel rows of cilia, the two rows being separated at their bases but fused at their tips. The endoplasm of *Spirostomum* is distinctly vacuo-

*Based upon Bishop's[9] description.

lated. The vacuoles are easily seen in living specimens. The animal can ingest protozoa as large as *Chilomonas paramecium,* therefore the food vacuoles of *Spriostomum* may contain small protozoa, as well as bacteria.

Descriptions of micrographs. The demarcation between ectoplasm and endoplasm is shown in FIGURES 2 and 3. The complex nature of ectoplasm is shown in FIGURES 3, 4, and 5. In these figures, note the multiplicity of intertwining fibrils, and sections through the basal bodies and myonemes.

Fibrils appear to be one of the dominant features of the ectoplasm. This deduction would seem to be logical, and easily arrived at without the aid of electron microscopical studies. So far as *Spirostomum's* structure is concerned, however, the micrographs accompanying this paper seem to be the first of their kind. They accordingly supply visible evidence in support of the deduction mentioned above.

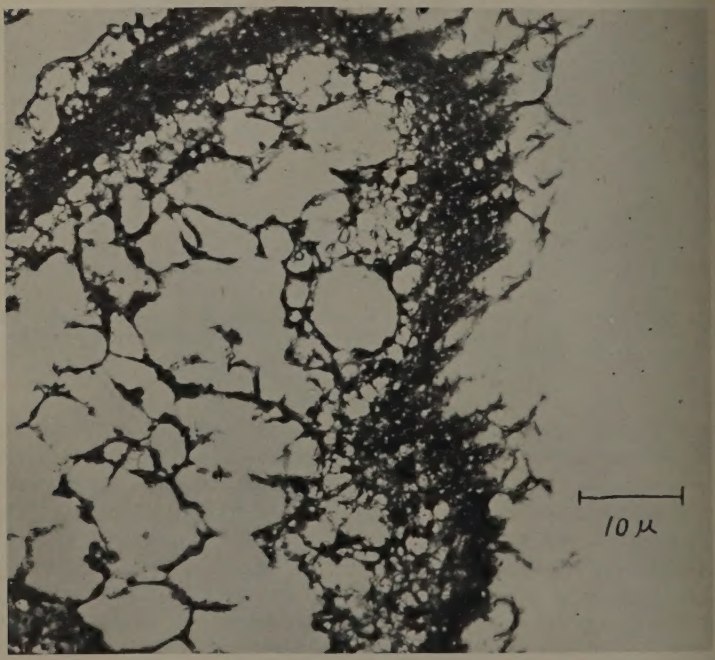

FIGURE 4. Intertwining ectoplasmic fibrils and ovoid myonemes in a tangential section. Portion of a food vacuole in lower left margin. Peristomial membranelles extending from ectoplasmic ridges. Randolph's fixation.

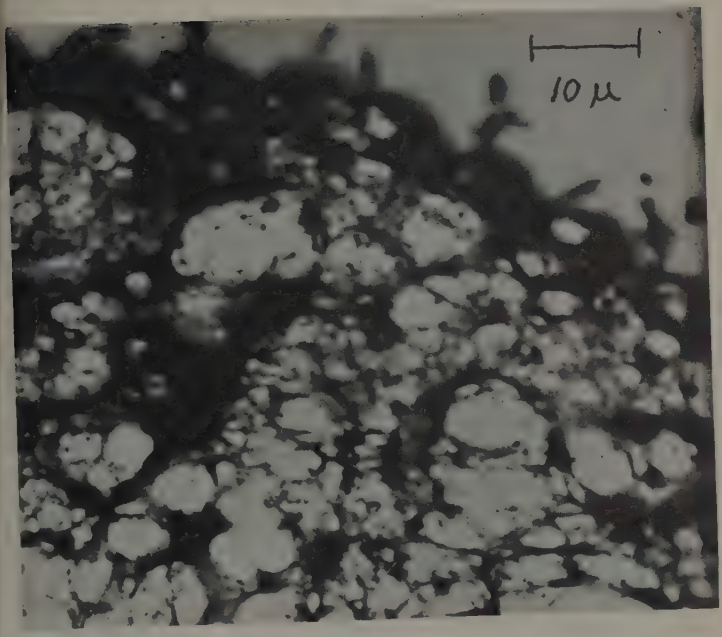

FIGURE 5. Ectoplasm and endoplasm after fixation in 2 per cent osmic acid solution. This electron micrograph was made with the RCA-type EMT electron microscope. Other micrographs illustrating this paper were made with the RCA-type EMU electron microscope.

The apparent stretching of some of the ectoplasmic fibrils raised the question as to the natural state of such fibrils in the living organism. Assuming that the visible evidence is good, the stretching suggests that the fibrils are endowed with the property of elasticity. This property would seem to account for the remarkable power of contraction that is so characteristic of living *Spirostoma*.

Particularly instructive is FIGURE 6, showing a longitudinal section through a locomotory cilium. The fibrillar organization of the cilium is clearly evident in the original micrograph. Jakus and Hall[13] published an account of "ultramicroscopic fibrils" in the cilia of *Paramecium*, an account that supplied the first electron-microscopical evidence of the compound nature of protozoan cilia. Our findings seem to be in agreement with the findings of Jakus and Hall in showing that *Spirostomum's* cilia consist of longitudinally arranged submicroscopic fibrils. These findings

may suggest that the cilia of other protozoa are composed of submicroscopic fibrils.

We have not succeeded in estimating the number of fibrils in a cilium, but such an estimate is not impossible with electron microscopy. According to the publication of Jakus and Hall, cited above, about 11 fibrils compose the cilium of *Paramecium*. Our original micrographs seem to show more than 11 submicroscopic fibrils in *Spirostomum's* cilium. From this finding, it may be inferred that the submicroscopic structure of cilia differs from one protozoan to another. It would be interesting to know whether there are characteristic differences in the numbers of ciliary fibrils in the different kinds of protozoa.

In FIGURE 3, particulate structure is revealed throughout the endoplasmic meshwork. Among these particles there is considerable variation in size. This variation may be due to their inherent composition or organization, or, perhaps, it may be due to the fact that the particles have different orientations. The possibility that they are artifacts is, of

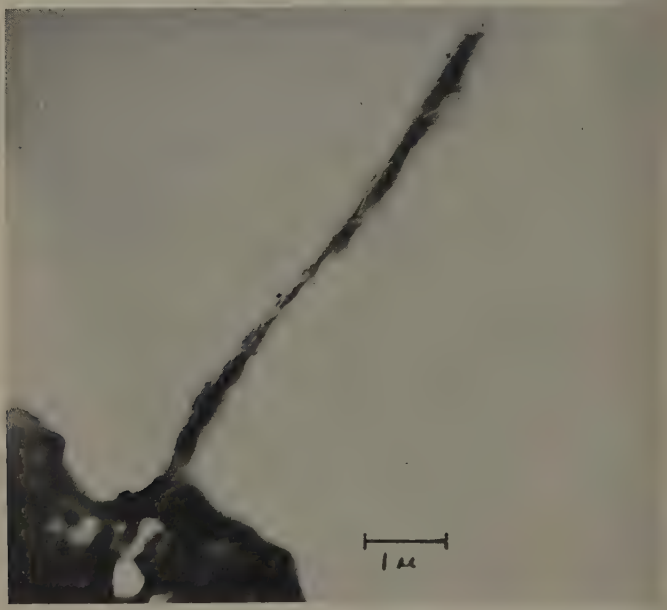

FIGURE 6. Longitudinal section of locomotory cilium revealing its submicroscopic fibrils. Bouin's fixation. Chromium shadowed (4:1).

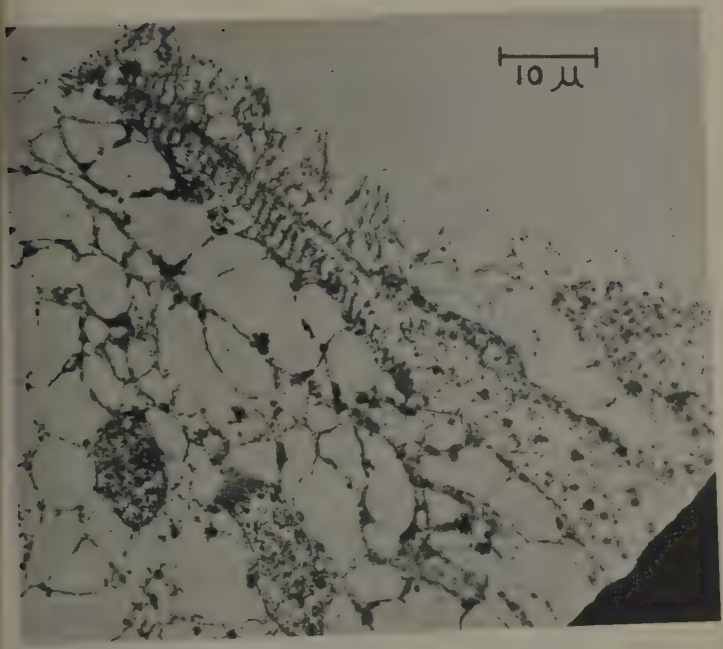

FIGURE 7. Oblique section showing two ovoid portions of macronucleus, endoplasm, and "platoons" of basal granules of peristomial membranelles. Randolph's fixation.

course, very real. In the living animal, endoplasm appears to be a homogenous continuum, but fixation seems to change the continuum to a reticulated coagulum. Therefore, a final interpretation of the submicroscopic pattern of *Spirostomum*'s cytoplasm must not be based upon the evidence now at hand. Instead, even the tentative interpretations must be postponed until some time in the future.

Prominent in the cytoplasm in FIGURES 3 and 8 are ovoid bodies that are relatively transparent to the electron beam. For the most part, these ovoid bodies are in contact with the cytoplasmic meshwork. Seeing these bodies for the first time, the author was inclined to identify the ovoids as artifacts resulting from the resin embedding method, and that interpretation may be correct. Then, however, it was discovered that one of our laboratory cultures contained numerous Spirostoma which were harboring a green symbiont whose identity was not determined. There-

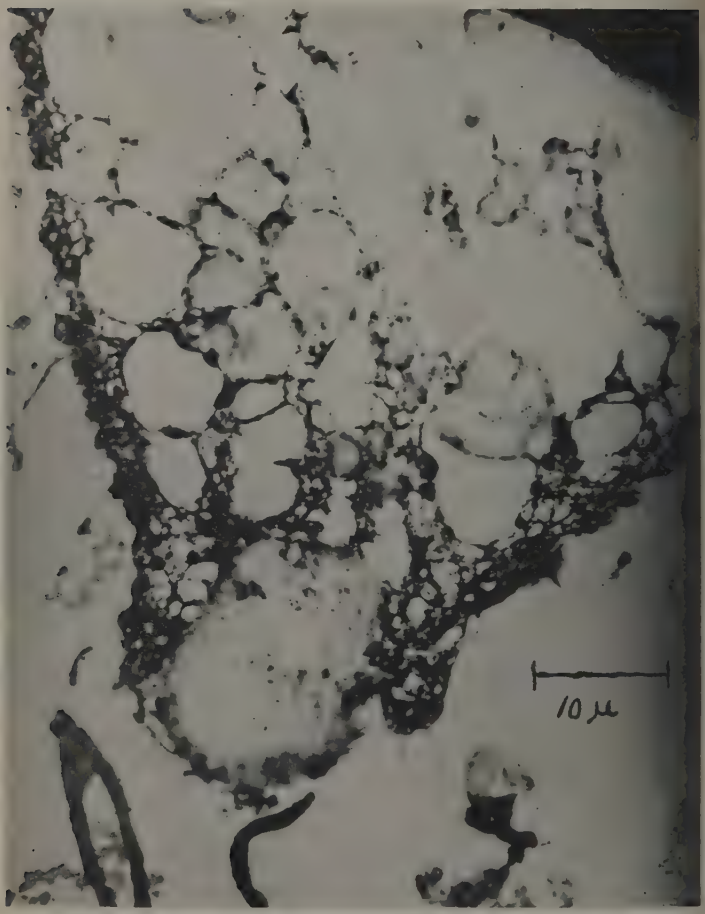

FIGURE 8. Longitudinal section of "spent" food vacuole being ejected from the cytopyge. Transparent ovoid bodies in endoplasm are symbionts.

fore, we suspect that the ovoid bodies that have been mentioned above are not artifacts. This suspicion is supported by the fact that these ovoids are strikingly absent from the medium surrounding the animals. The large "spent" food vacuole being evacuated through the cytopyge in FIGURE 8 should be noted.

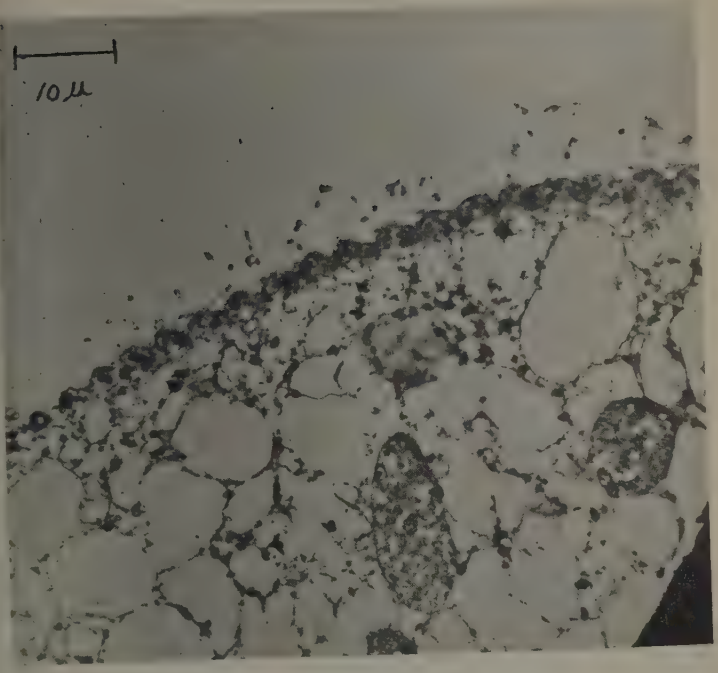

FIGURE 9. Three portions of the macronucleus and one portion of a food vacuole. Randolph's fixation.

Some visualization of the ultrastructure of the macronucleus is possible in FIGURES 3, 9, 10, and 11. Clearly evident is the particulate nature of the macronucleus, and also its opacity with reference to the electron beam. In FIGURES 10 and 11, the structural heterogeneity of the macronucleus is very strikingly shown by the chromium-shadowing technique. These micrographs reveal particles whose size is of the order of approximately 2,600 Å. The images revealed in these figures are accepted with a critical attitude. Nevertheless, the structures revealed actually exist, although they may be somewhat altered by the procedure for preparation.

In FIGURE 7, the most instructive details are revealed in the "platoons" of basal granules located in the upper left portion of the figure. This specimen was sectioned obliquely; therefore, one sees here the equivalent of a serial section analysis of the rows of basal granules from

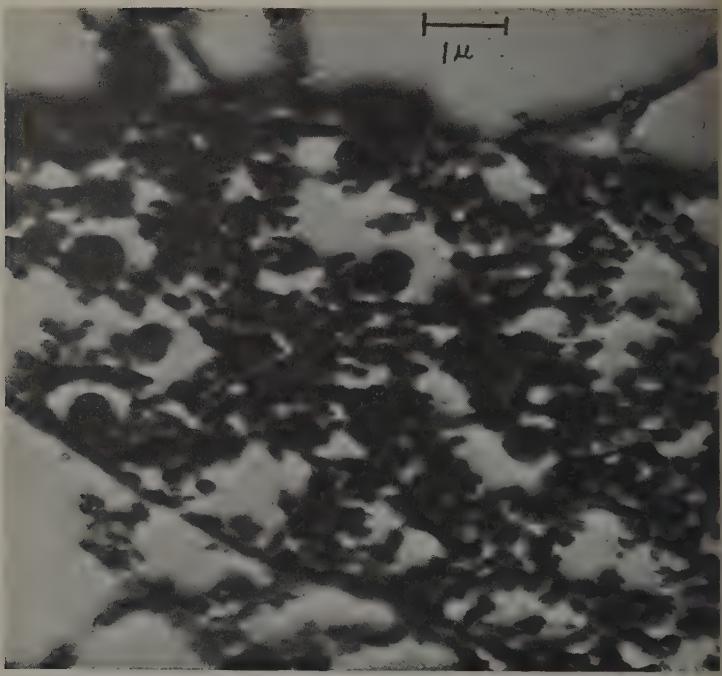

FIGURE 10. Chromium shadowed section through a portion of the macronucleus. Randolph's fixation.

which the peristomial cilia spring. It can be seen that the basal granules are intimately connected with each other by fine structures, probably fibrillar in organization; these intimate connections most certainly account for the high degree of synchronism which characterizes the wave-like beat of the peristomial membranelles. Support for this interpretation of the peristomial basal granules is given in FIGURE 13.

Discussion

The observations reported in this paper support the view that electron microscopy is applicable to many protozoological problems, particularly to problems concerning the structural organization of the protozoan cell. By means of electron microscopy, we can expect to discern fine details that were previously unrecognized, or only vaguely suspected. For example, it would be very difficult to describe the complex nature of *Spirostomum's* ectoplasm with less optical resolution

han that provided by the electron microscope, as the compound nature of cilia would not be revealed with less optical resolution. Despite the problems and difficulties inherent in fixation, or embedding, or in any of the other technical procedures, concepts concerning the Protozoa may be re-examined in the light of newer knowledge that can be obtained through electron microscopy.

The problem of fixation is one that every cytologist recognizes as being worthy of careful consideration. The difficulties of fixation are magnified in electron microscopy, perhaps in proportion to the gain in optical resolution. An ideal fixing fluid, at least one that can accomplish all the purposes of excellent fixation, seems not to have been developed at the present time. An ideal fixative would be capable of performing three functions: first, it should prevent morphological alteration from

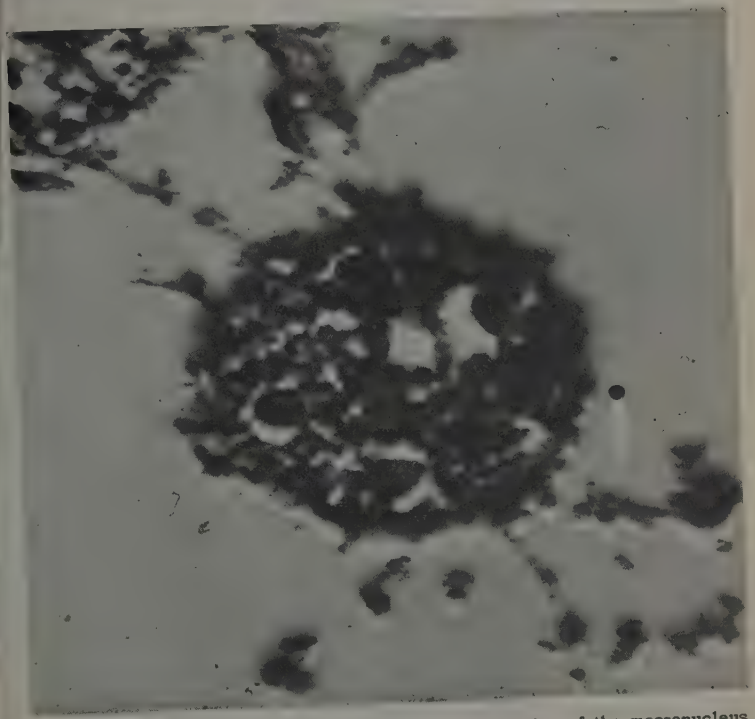

FIGURE 11. Chromium shadowed section of a portion of the macronucleus. Polystyrene latex particle, approximately 2,600 Å, at the right, affords a basis for calibration. Randolph's fixation.

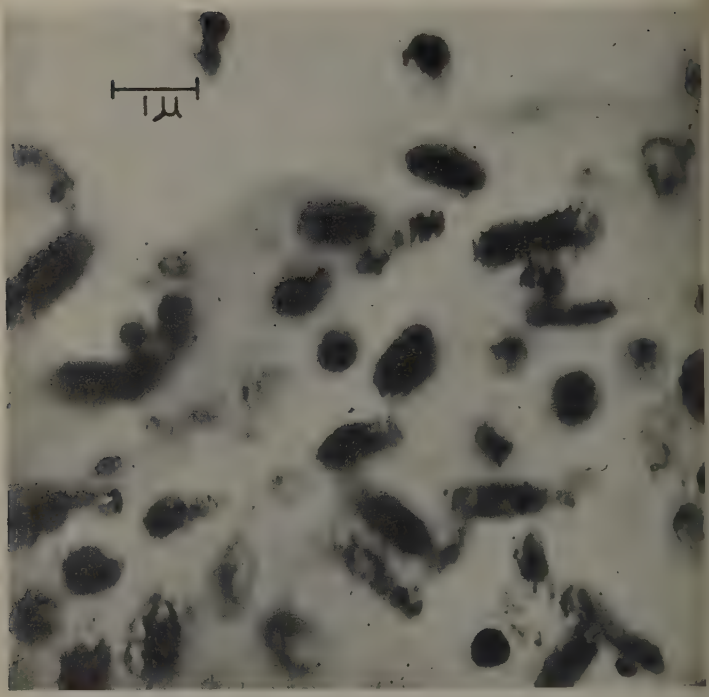

FIGURE 12. Chromium shadowed section of microorganisms in a food vacuole. Bouin's fixation.

the moment when the cell is placed in the fixing fluid; second, it should alter the chemistry of the cell so that the cell may be dehydrated and embedded without being dissolved or distorted; and, third, it should yield preparations equally suitable for examination and study with the optical microscope and the electron microscope. Wyckoff,[14] Dalton,[15] Birbeck,[16] and many other investigators have discussed the deficiencies of fixatives, especially in so far as the deficiencies are revealed by the resolving powers of the electron microscope. According to the publications of the investigators named above and on the basis of the experience gained during the progress of this study, it is suggested that the choice of a fixative for electron microscopical studies must be determined by the kind of tissues or organisms to be examined, and by the cellular

mponents under consideration. The fixatives that are satisfactory for l cytological examination of *Spirostomum* may not be appropriate for different organism. Likewise, fixatives that are suitable for *Spiro-omum*'s macronuclei may not be suitable for this animal's myonemes. his may account for the fact that fine details are not revealed in our iginal electron micrographs of basal bodies (FIGURE 13), although ch details are visualized in the thin sections of cilia and macronuclei IGURES 6 and 11).

Summary

An electron microscopical study of thin sections of *Spirostomum mbiguum* is presented. The Protozoa were infiltrated with n-butyl ethacrylate, embedded in the polymerized monomer, and sectioned ccording to the thermal expansion method that was developed at the

FIGURE 13. Chromium shadowed basal granules of peristomial membranelles. Randolph's fixation.

National Bureau of Standards by Newman, Borysko, and Swerdlow. The accompanying text, figures, and numerous unreproduced micrographs reveal fine structures that have not been resolved previously with ordinary optical microscopy. Cilia, macronuclei, and other cellular components are described. Some attention is given to the subject of fixation. It is stated that, from a practical point of view, the choice of fixing solutions employed in electron microscopy must be governed by the particular detail that one desires to preserve.

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